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## Graphical Abstract

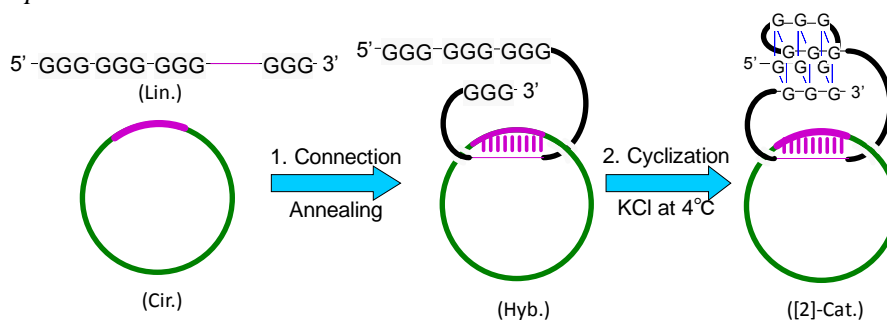
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### Single Strand DNA Catenane Synthesis using the formation of G-Quadruplex Structure

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# Single Strand DNA Catenane Synthesis Using the Formation of G-Quadruplex Structure

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## ABSTRACT

DNA is a good material for constructing nanostructures such as DNA origami. One of the challenges in this field is constructing a topologically complex structure. Here, we synthesized a DNA catenane through the formation of a G-quadruplex structure. The formation of the DNA catenane was investigated by gel electrophoresis. Interestingly, the synthesized DNA catenane was destroyed by heat treatment. Because conventional methods to construct DNA catenane include enzymatic ligation or chemical reactions, DNA is cyclized by covalent bond connection and never destroyed by heat treatment. To our knowledge, this is the first report of the synthesis of DNA catenane without using covalent bonds. Our novel way of synthesizing DNA catenane may be of use in easily recoverable DNA topological labeling.

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## 1. Introduction

Constructing topologically interesting molecular architectures is of great interest because architectures such as catenanes, rotaxanes, and knots show unique physical properties.<sup>1</sup> These have been engineered successfully using organic and supramolecular synthetic methods.<sup>2–4</sup> Some of these unique architectures are now being investigated and applied as molecular switches or molecular motors.<sup>5–9</sup>

In the nucleic acid world, DNA is a promising material for constructing nanomaterials because of its complementary self-assembly. Various DNA-based nanostructures have been reported, such as DNA origami,<sup>10</sup> rotaxanes,<sup>11</sup> Borromean rings,<sup>12</sup> octahedrons,<sup>13</sup> knots,<sup>14</sup> and catenanes.<sup>15</sup> Among them, DNA catenane is one of the most attractive architectures because it exists in biological contexts such as mitochondria in human cells.<sup>16,17</sup> Probably the most common occurrence of DNA catenane is as an intermediate during the replication of circular DNA molecules, which have been observed in various systems including bacterial plasmids<sup>18–20</sup> and the animal virus SV40.<sup>21</sup>

Many studies have been reported on DNA catenane synthesis because of its potential use in DNA topological labeling.<sup>22,23</sup> The synthesis of single strand DNA catenane is achieved by two steps: connection of two DNA strands and cyclization of the DNA strand(s). The enzymatic template-directed ligation of oligonucleotides is used widely for cyclization.<sup>24–27</sup> Other connect

approaches such as photocrosslinking and chemical reactions to the end of oligonucleotides have been reported.<sup>28–31</sup> However, these methods still have a laborious introduction of modified bases. In this study, G-quadruplex formation was used in the cyclization to avoid the introduction of modified bases.

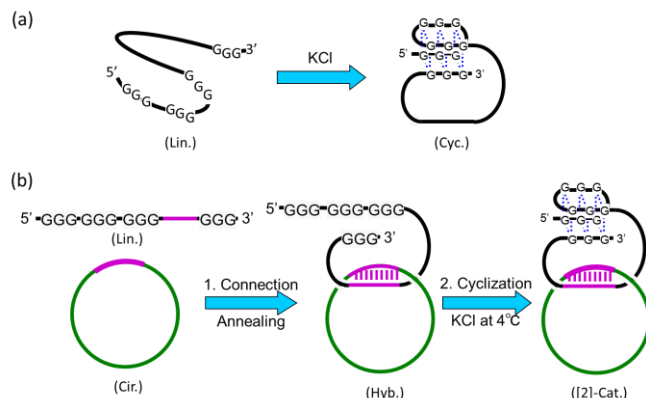
A G-quadruplex structure is a four-stranded oligonucleotide formation observed in G-rich sequences. Because the G-quadruplex formation is highly stabilized by potassium ions, it could be a trigger for the formation of the G-quadruplex structure. Various DNA switches have been developed, such as DNA logic gates,<sup>32,33</sup> cation detectors,<sup>34,35</sup> and self-assembly of nanoparticles.<sup>36</sup> We recently observed directly the dynamics of G-quadruplex formation switched by potassium ions on a DNA nanoscaffold.<sup>37</sup> We also demonstrated the cyclization of DNA based on the formation of a G-quadruplex structure (Figure 1a).<sup>38</sup> In this study, we first investigated the stability of cyclized DNA created by G-quadruplex formation to customize the sequence for the synthesis of DNA catenane. We then confirmed the formation of DNA catenane by PAGE analysis.

## 2. Results and discussion

### 2.1. Stability of loop formation

As described above, DNA catenane synthesis involves two steps: connection and cyclization. Our strategy for catenane synthesis is shown in Figure 1b. The initial connection step was

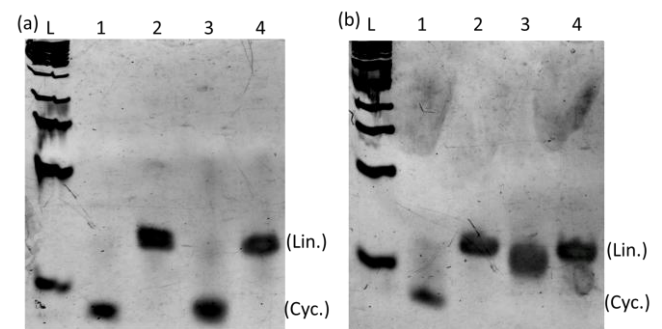
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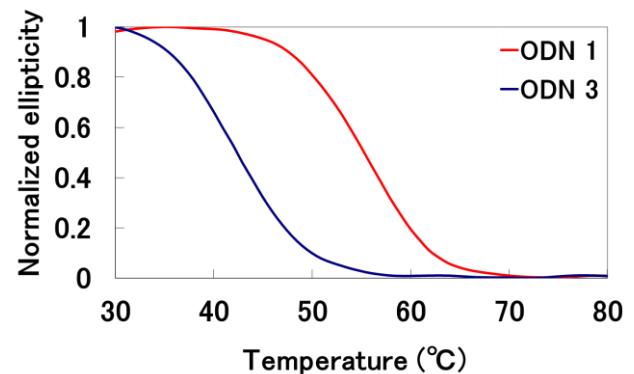
**Figure 1.** (a) Schematic representation of DNA cyclization through G-quadruplex formation. The linear oligonucleotide (Lin.) was cyclized by the addition of KCl. (b) Schematic representation of the strategy for DNA catenane synthesis. The first step is to connect the circular oligonucleotide (Cir.) and linear oligonucleotide (Lin.) using an annealing process. The second step is the cyclization of hybridized oligonucleotides (Hyb.) through the formation of the G-quadruplex structure triggered by the addition of KCl.

implemented by annealing with complementary strands, and the second cyclization step was performed by the formation of a G-quadruplex triggered by the addition of potassium ions. Because the cyclization step is performed after the annealing process, the formation of the G-quadruplex should be performed at low temperature to avoid the dissociation of the two strands. In other words, the desired G-quadruplex structure is unstable at low temperature without potassium ions but is highly stable in the presence of potassium ions.

The sequences of oligonucleotides used in this study are shown in Table 1. We prepared oligonucleotides containing G-rich sequences at both ends. For comparison, their mutant oligonucleotides, which cannot form G-quadruplex structures, were also prepared. ODN 1 contained three G-tracts at one end and one G-tract at the other end and should form a [3+1] hybrid type G-quadruplex structure.<sup>38</sup> ODN 3 contained two G-tracts at both ends and should form a [2+2] type G-quadruplex structure. The loop formation was investigated by PAGE analysis



**Figure 2.** PAGE analysis of ODNs 1–4 (lanes 1–4) (a) at 4°C and (b) at room temperature. Cyc.=cyclized; Lin.=linear. 100 ng oligonucleotides sample were loaded for the electrophoresis.

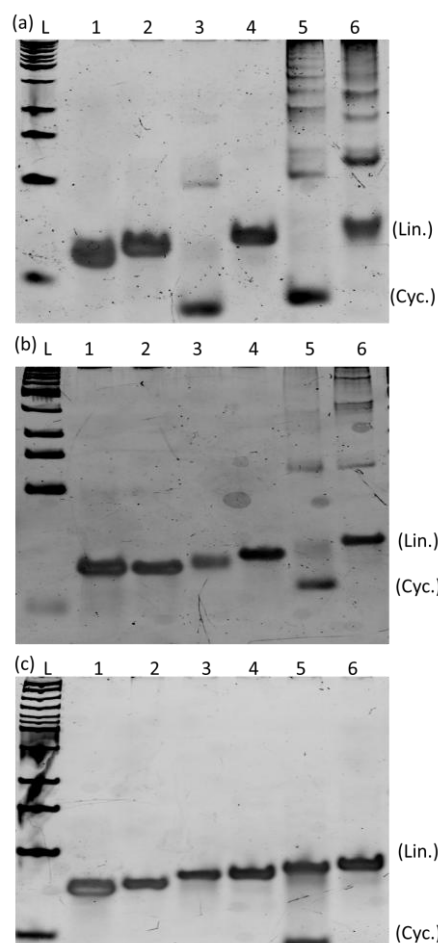


**Figure 3.** CD melting curves of ODNs 1 and 3 monitored at 290 nm. The  $T_m$  was 55.8°C for ODN 1 and 42.2°C for ODN 3. 5  $\mu$ M oligonucleotides were used for measurements.

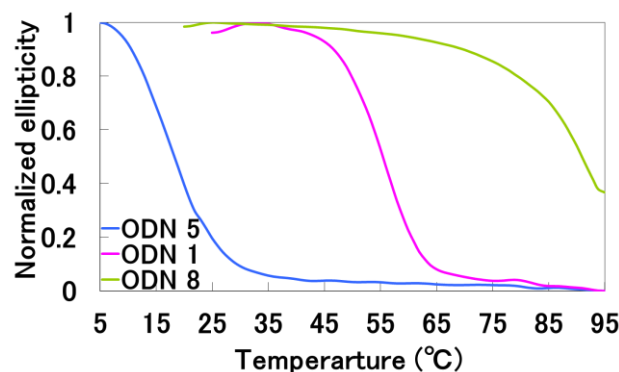
containing potassium ions (Figure 2a). Clearly, ODNs 1 and 3 showed faster migrating bands at 4°C compared with their mutant oligonucleotides ODNs 2 and 4. This result indicated that ODNs 1 and 3 formed a compact structure through the G-quadruplex formation. Previously, we showed that the  $T_m$  of ODN 1 did not depend on the concentration of oligonucleotide, indicating that ODN 1 formed intramolecular G-quadruplex structures.<sup>38</sup> Interestingly, the mobility of ODN 3 was the same as that of ODN 4 when they were electrophoresed at room temperature (Figure 2b). This suggests that the G-quadruplex

**Table.1** Sequences of oligonucleotides used in this study. The 10 bp complementary regions are underlined

Name	Sequence
ODN 1	5'-GGGTTAGGGTTAGGG-T <sub>21</sub> -GGG-3'
ODN 2	5'-GAGTTAGGGTTAGGG-T <sub>21</sub> -GGG-3'
ODN 3	5'-GGGTTAGGG-T <sub>21</sub> -GGGTTAGGG-3'
ODN 4	5'-GAGTTAGGG-T <sub>21</sub> -GGGTTAGGG-3'
ODN 5	5'-GGTTAGGTTAGG-T <sub>21</sub> -GG-3'
ODN 6	5'-GATTAGGTTAGG-T <sub>21</sub> -GG-3'
ODN 7	5'-GAGTTAGGGTTAGGG-T <sub>21</sub> -GGG-3'
ODN 8	5'-GGGGTTAGGGTTAGGGG-T <sub>21</sub> -GGGG-3'
ODN 9	5'-GAAGTTAGGGTTAGGGG-T <sub>21</sub> -GGGG-3'
ODN 10	5'-TCCGACTTTTGTCTGGATCCACG-T <sub>32</sub> -CGATATCGCACGTACATTCGTGGA-3'
ODN 11	5'-GGGTTAGGGTTAGGG-T <sub>10</sub> -TACGTGCGAT-T <sub>10</sub> -GGGT-3'
ODN 12	5'-TCCGACAGGCCTAATG-T <sub>32</sub> -GTCGGATCCACGATCGCACGTA-T <sub>32</sub> -CGTGGA-3'
ODN 13	5'-GGGTTAGGGTTAGGG-T <sub>10</sub> -CATTAGGCCT-T <sub>10</sub> -GGGT-3'
ODN 14	5'-GAGTTAGGGTTAGGG-T <sub>10</sub> -TACGTGCGAT-T <sub>10</sub> -GGGT-3'
ODN 15	5'-GAGTTAGGGTTAGGG-T <sub>10</sub> -CATTAGGCCT-T <sub>10</sub> -GGGT-3'



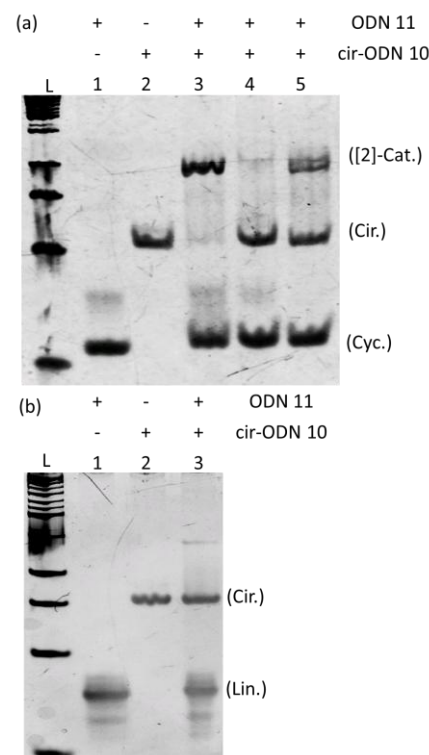
**Figure 4.** PAGE analysis (a) at 4°C, (b) at 60°C, and (c) at 4°C without potassium ions. Lane 1, ODN 5; lane 2, ODN 6; lane 3, ODN 1; lane 4, ODN 7; lane 5, ODN 8; lane 6, ODN 9. 100 ng oligonucleotides sample were loaded for the electrophoresis.



**Figure 5.** CD melting curves of ODNs 1, 5, and 8 monitored at 290 nm. The  $T_m$  values of ODNs 1, 5, and 8 were 55.8°C, 17.3°C, and 91.4°C, respectively. 5  $\mu$ M oligonucleotides were used for measurements.

structure of ODN 3 is not as stable as that of ODN 1. This result is consistent with the melting curves of ODNs 1 and 3 (Figure 3). Because the stable G-quadruplex structure is better for catenane synthesis in the presence of potassium ions, the [3+1] hybrid G-quadruplex was selected for DNA catenane synthesis.

Next, we prepared ODNs 1, 5, and 8 containing different numbers of guanine bases in the G-tract to customize its length (Table 1). ODNs 6, 7, and 9 were mutant oligonucleotides used as negative controls for the G-quadruplex formation. PAGE analysis showed that the mobilities of ODNs 5 and 6 were the same at 4°C (Figure 4a). This result indicates that the



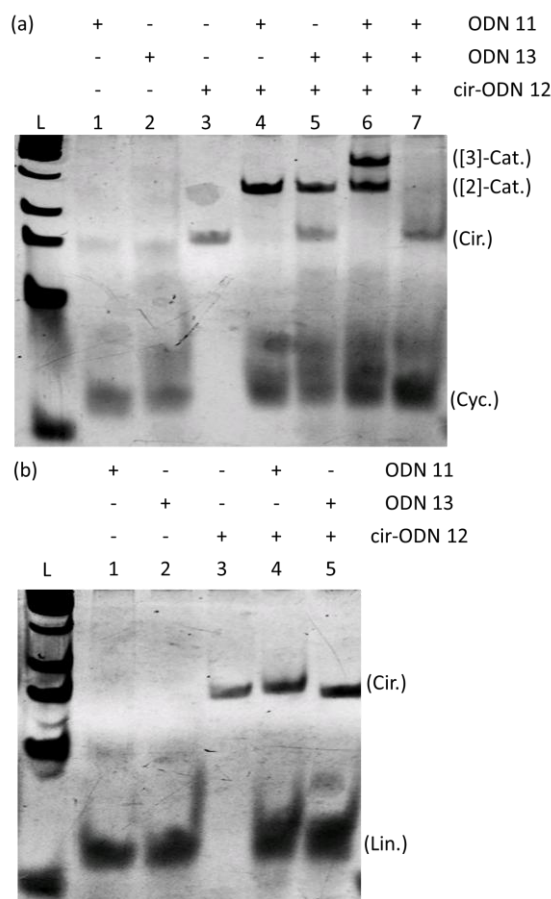
**Figure 6.** PAGE analysis (a) in the presence of potassium ions and (b) in the absence of potassium ions. (a) Lane 1, ODN 11; lane 2, circular ODN 10; lane 3, [2]-catenane composed of circular ODNs 10 and 11; lane 4, unconnected circular ODNs 10 and 11; lane 5, heat-treated mixture of circular ODNs 10 and ODN 11 after synthesis of [2]-catenane. (b) Lane 1, ODN 11; lane 2, circular ODN 10; lane 3, mixture of circular ODNs 10 and 11. The amount of oligonucleotides were 60 ng and 90 ng for circular ODN 10 and ODN 11, respectively.

G-quadruplex structure comprising two continuous guanine bases was unstable even at 4°C. In Figure 4a, ODN 8 shows several slow migrating bands, which indicate the existence of dimers or multimers of ODN 8 created through intermolecular interaction. Interestingly, ODN 8 showed a faster migrating band compared with its mutant oligonucleotide ODN 9 even at 60 °C (Figure 4b). In addition, ODN 8 also showed a faster migrating band even in the absence of potassium ions at 4°C (Figure 4c). These results clearly indicate that the G-quadruplex structure of ODN 8 is too stable for catenane synthesis. The melting curves of ODNs 1, 5, and 8 are consistent with the results mentioned above (Figure 5). Based on these results, we conclude that the [3+1] hybrid G-quadruplex structure containing three G-tetrads is a good G-quadruplex structure for the synthesis of DNA catenane.

## 2.2. Synthesis of [2]-catenane

We prepared a circular oligonucleotide, ODN 10, and a linear oligonucleotide containing G-tracts at the ends, ODN 11, for the synthesis of DNA catenane (Table 1). The circular ODNs 10 and 11 contained a 10 bp complementary region for the connection step of annealing. After annealing the mixture of circular ODNs 10 and 11, KCl was added at 4°C to form the G-quadruplex structure. The formation of DNA catenane was investigated by PAGE analysis (Figure 6a). A slow migrating band appeared in lane 3, indicating the existence of DNA catenane. To confirm further the DNA catenane formation, circular ODN 10 was added to cyclized ODN 11; that is, ODN 10 was added to circular ODN 11 after KCl was added to induce cyclization. In this case, DNA catenane could not be formed because ODN 11 was cyclized before circular ODN 10 was connected to ODN 11 (lane 4 in





**Figure 7.** PAGE analysis of circular ODNs 12 and 11–15. (a) Lane 1, ODN 11; lane 2, ODN 13; lane 3, circular ODN 12; lane 4, [2]-catenane comprising circular ODNs 12 and 11; lane 5, [2]-catenane comprising circular ODNs 12 and 13; lane 6, [3]-catenane comprising circular ODNs 11, 12, and 13; lane 7, unconnected circular ODNs 11, 12, and 13. (b) Lane 1, ODN 14; lane 2, ODN 15; lane 3, circular ODN 12; lane 4, mixture of circular ODNs 12 and 14; lane 5, mixture of circular ODNs 12 and 15. The amount of oligonucleotides were 90 ng, 60 ng, and 90 ng for ODN 11, circular ODN 12, and ODN 12, respectively.

Figure 6a). Interestingly, part of DNA catenane was destroyed by heat treatment (lane 5 in Figure 6a). This result indicates that the synthesized DNA catenane was clipped by weak hydrogen bonds.

Next, the oligonucleotides were electrophoresed in the absence of potassium ions to investigate the effect of G-quadruplex formation on catenane synthesis. Figure 6b shows clearly that there was no DNA catenane in the gel. This result indicates that the formation of G-quadruplex structure is a key step in the synthesis of DNA catenane. More noteworthy is that this result rules out the existence of the hybridized structure (see Hyb. in Figure 1b) between circular ODNs 10 and 11 in the PAGE condition. In other words, linear or cyclized ODN 11 cannot interact with circular ODN 10 in the PAGE condition. The  $T_m$  values of the [3+1] G-quadruplex structure and the hybridized circular ODNs 10 and 11 were 55.8°C and 31.5°C, respectively. These data agree with the results of the PAGE analysis in Figure 6.

### 2.3. Synthesis of [3]-catenane

We explored the method for synthesizing [3]-catenane. We synthesized circular oligonucleotide, ODN 12, which was complementary to linear oligonucleotides, ODNs 11 and 13

(Table 1). Using a similar method, three oligonucleotides ODN 11, circular ODN 12, and ODN 13 were mixed together following annealing without potassium ions. KCl was then added to the mixture at 4°C to cyclize ODNs 11 and 13. PAGE analysis showed clearly a slower migrating band, which indicated the formation of [3]-catenane (lane 6 in Figure 7a). For comparison, ODNs 11 and 13 were cyclized independently by addition of KCl. These cyclized ODNs were mixed with circular ODN 12 at 4°C and analyzed by PAGE (lane 7 in Figure 7a). No slower migrating bands appeared, indicating the absence of DNA catenane.

To confirm that the cyclization was triggered by the formation of G-quadruplexes, we prepared mutant oligonucleotides ODNs 14 and 15, which cannot form the G-quadruplex structure. PAGE analysis of these oligonucleotides did not show any slow migrating bands, indicating that the G-quadruplex formation was a key step in the DNA catenane synthesis (Figure 7b).

## 3. Conclusion

Single strand DNA catenanes were synthesized via G-quadruplex formation triggered by the addition of potassium ions. The formation of catenane was confirmed by gel electrophoresis. Interestingly, the synthesized DNA catenane was destroyed easily by heat treatment because of decomposition of the G-quadruplex structure. The conventional methods for synthesizing DNA catenane include the connection of covalent bonds. To our knowledge, this is the first report to achieve the synthesis of DNA catenane without using enzymes or chemical reactions. Our novel strategy for DNA catenane synthesis has great potential for easily recoverable DNA topological labeling.

## 4. Experimental

### 4.1. Materials

All oligonucleotides used in this study are listed in Table 1. The oligonucleotides were purchased from Sigma Aldrich Japan. Those oligonucleotides were used without further purification.

### 4.2. PAGE analysis

Samples were heated at 95 °C for 10 min, followed by gradual cooling to 4 °C unless otherwise stated. Then samples were electrophoresed in 10% polyacrylamide gels containing 50 mM Tris-HCl (pH 7.0) and 25 mM  $K_2B_4O_7$ . 10% polyacrylamide gel containing 1XTBE was used for potassium deficient condition. The gels were stained with SYBR Gold and observed with FUJIFILM LAS-3000UV mini.

### 4.3. $T_m$ measurements by CD spectroscopy

The CD spectra were measured using an AVIV MODEL 62 DS/202 CD spectrometer. A quartz cell of 0.1 cm optical path length. The buffer used for CD was 50 mM Tris-HCl (pH 7.0) and 25 mM  $K_2B_4O_7$ . 5  $\mu$ M oligonucleotides samples were heated at 95 °C for 10 min and gradually cooled to 4°C. The melting curves were obtained by monitoring a 290 nm CD band with increasing temperature at 1°C/min.

### 4.4. Preparation of circular oligonucleotides

The circular ODN 10 and ODN 12 were synthesized by DNA template-directed synthesis.<sup>31</sup> These oligonucleotides were ligated with Ligation-Convenience Kit (NIPPON GENE, Tokyo) at 16°C for 30 min. The reaction was stopped by heat inactivation, followed by denaturing PAGE and native PAGE purification.

### 4.5. Synthesis of DNA catenane

The circular oligonucleotide and its complementary G-quadruplex forming oligonucleotide(s) were mixed in 50 mM Tris-HCl (pH 7.0) and heated at 95°C for 10 min, followed by gradual cooling to 4°C. After annealing, 1 M KCl was added to the reaction mixture. The formation of catenane was investigated by PAGE analysis without any purification.

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